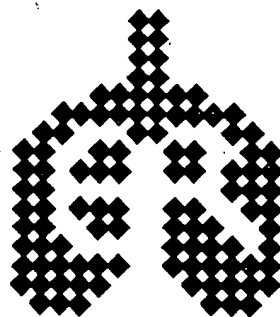


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Pulmonary immune cells in health and disease: the eosinophil leucocyte (Part II)

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ABSTRACT: The second part of this review on eosinophils focuses on biological cell functions and surveys the various deleterious mechanisms involved in the eosinophil-dominated inflammatory reaction. It discusses the possible pathogenic role of eosinophils in several eosinophil-related diseases, such as parasitic infections, interstitial lung disorders and bronchial diseases, graft rejection, vasculitic granulomatous disorders, pleural effusion, and bronchogenic tumours.

The final section of the article highlights the possible recent pharmacological and future therapeutic approaches in modifying eosinophil recruitment and function.

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Biological cell functions

The eosinophil mediates its effector functions through various distinct cellular mechanisms, namely nonoxidative, oxidative, and humoral mechanisms (fig. 7).

Nonoxidative mechanisms

As mentioned previously, the three basic proteins as well as eosinophil peroxidase (EPO) have toxic properties independent of molecular oxygen species. For instance, major basic protein (MBP) [323], eosinophil cationic protein (ECP) [324] and EPO in the presence of hydrogen superoxide and a halide [99] kill helminthic parasites *in vitro*. In addition, these proteins are toxic for tumour cells and/or mammalian cells [325], including human pulmonary parenchyma and interstitial matrix [326, 327], cultured human lung epithelial cells [328], and guinea-pig respiratory epithelium [252, 253, 329]. Furthermore, it has been demonstrated that MBP damages human bronchial epithelium, which consists of desquamation and destruction of ciliated cells [329]. Finally, release of their granule proteins by platelet-

activating factor (PAF)-activated intact eosinophils has been shown to imitate the characteristic pathological findings of airway epithelium in asthma [330].

In addition to their cytotoxic action, eosinophil cationic proteins at certain subtoxic concentrations also stimulate other inflammatory cells. For instance, MBP and ECP have been shown to degranulate platelets [305], induce histamine release from mast cells and basophils [240, 245, 331], as well as superoxide anion generation and lysosomal enzyme release by human neutrophils [264].

Finally, eosinophil proteins may affect airway muscle function and hyperresponsiveness. Recent reports investigating the effects of direct intratracheal instillation of purified eosinophil granule proteins on pulmonary function and airway responsiveness in primates [281, 332] have shown that MBP induces a dose-related increase in airway responsiveness to inhaled methacholine. In the same model, both MBP and EPO caused a transient bronchoconstriction immediately after instillation, that resolved within 1 h. Interestingly, other eosinophil granular proteins failed to effect airway responsiveness or pulmonary function.

Mode of action. The mode of action of these basic proteins is not yet fully established. Structurally, they consist of: 1) charged domains made up predominantly of basic amino acids, such as lysine and arginine [333-335]; and 2) hydrophobic amino acid chain segments [336].

*References [1] - [322], Figures 1-6 and Tables 1-6 are to be found in Part I.

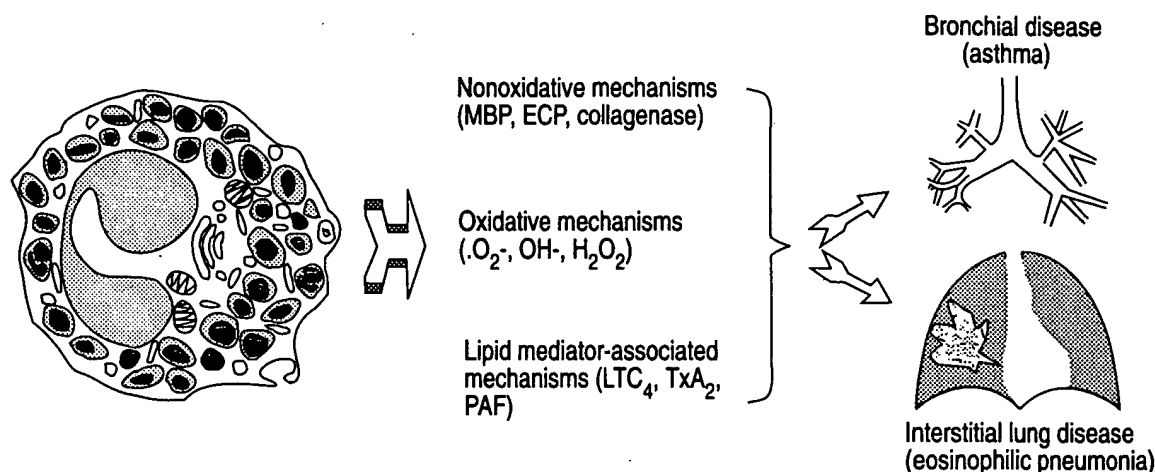


Fig. 7. — Nonoxidative, oxidative and lipid mediator-associated effector functions of eosinophil leucocytes on bronchial and interstitial tissue. MBP: major basic protein; ECP: eosinophil cationic protein; LTC_4 : leukotriene C_4 ; TxA_2 : thromboxane A_2 ; PAF: platelet-activating factor.

Thus, their physicochemical properties resemble that of hymenoptera venom toxins such as melittin or mastoporan [174, 337, 338], the ninth component of complement [339], and perforin 1 from natural killer and cytotoxic T-lymphocytes [340] as well as bacterial streptolysin-O and staphylococcal α -toxin [341].

These proteins have in common a high affinity for plasma membranes, to which they bind in two distinct stages: firstly the proteins approach the cell through the formation of electrostatic bonds between their positively charged residues and the invariably negatively charged external membrane surface due to its content of acidic phospholipids, glycolipids and glycoproteins [342, 343]. This interaction is then followed by insertion of the lipophilic molecule fragment into the membrane lipid bilayer. The driving force for the hydrophobic interaction may be the entropic advantage gained from desolvation of both the hydrophobic core of the phospholipid bilayer and the apolar surface of the amphiphilic protein [337, 344–346]. These protein-membrane interactions induce a number of disturbances in the target cell membrane including clustering of negatively charged components and aggregation of adjacent membranes [343]. These processes, in turn, may destabilize membranes, induce fusion of adjacent bilayers, activate phospholipase A_2 [131, 169, 347], and form voltage-insensitive transmembrane ion channels [237, 340]. Once enough protein has breached the bilayer, a cascade of events, such as osmotic imbalance and swelling [338], loss of cellular contents, or influx of Ca^{2+} , is likely to take place, which eventually leads to cell death and lysis [174, 337]. This mechanism of toxicity was first proposed in 1987 [337], and was recently confirmed using fluorescence and circular dichroism spectroscopy [348]. Additional efforts to further elucidate the mechanism are currently under way.

Oxidative mechanisms

Eosinophils may also exert their effector function by two oxygen-dependent mechanisms [111, 112, 330].

One involves the generation of toxic oxygen radicals ($1O_2$ and $\cdot O_2$), and the other, the production of hypobromous acid (HOBr), hydriodic acid (HIO $_3$), or hydrobromic acid (HBrO $_3$). In addition, newly formed superoxide anions spontaneously dismutates to H_2O_2 . Both $1O_2$ and H_2O_2 alone are likely to exert toxic effects [111]. In addition, in the presence of H_2O_2 EPO oxidizes the halides, iodide (I^-) or bromide (Br^-), to their corresponding hypohalous acids, which, in turn, are able to oxidize a wide range of target molecules in cells and micro-organisms. The EPO/ H_2O_2 /halide system is toxic to bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Legionella pneumophila*, and *Mycobacterium leprae*, as well as to fungi, the schistosomula of *Schistosoma mansoni*, the newborn larvae of *Trichinella spiralis*, *Trypanosoma cruzi* trypomastigotes, *Toxoplasma gondii*, tumour cells and mast cells (for review see [2, 349, 350]).

The eosinophil is exceptionally rich in peroxidase, and the enzyme is released both by soluble stimuli [73, 110, 131, 135, 169], and by adhesion to larger opsonized targets [2, 30, 33, 56, 159, 169, 208, 349, 350]. EPO is a highly basic protein that binds avidly to negatively charged surfaces with retention of peroxidase activity. In the presence of H_2O_2 and halide, target cells with surface bound EPO are rapidly destroyed, as has been demonstrated with *S. aureus*, *L. pneumophila*, *T. gondii*, *T. cruzi*, schistosomula of *S. mansoni*, and tumour cells [2, 99, 349, 350]. In addition, the toxic effect of intact neutrophils or macrophages is considerably increased when EPO is bound to the target cell, utilizing the H_2O_2 generated by the phagocyte more efficiently [97, 98, 349]. Finally, EPO can associate with mast cell granules to form a highly bactericidal and tumouricidal complex in the presence of H_2O_2 and a halide [232, 235, 236, 242].

Lipid mediator-mediated mechanisms

Eosinophils contribute to inflammation through the *de novo* generation and release of lipid mediators, such

as platelet-activating factor (PAF) [117, 124–126], leukotriene C_4 (LTC_4) [115, 117, 120], prostaglandin E (PGE) [118–122], prostaglandin F_1 (PGF_1) and thromboxane A_2 (TxA_2) [119–121, 123]. Using combined capillary gas chromatography/mass spectrometry, two more prostanoids, prostaglandin D_2 (PGD_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), have been shown to be generated by human and guinea-pig eosinophils [64, 73, 123], which may induce certain pathophysiological effects *in vivo*. Eosinophil-derived PAF, LTC_4 or TxA_2 , PGD_2 , and $PGF_{2\alpha}$, for instance, may contribute to tissue inflammation, bronchoconstriction or bronchial hyperreactivity in late-phase asthmatic reactions where eosinophils are characteristically found. PGE has been shown to down-regulate eosinophil function and may serve as a negative feedback signal for eosinophils [119].

Eosinophil-dominated inflammatory reaction

As outlined above, considerable circumstantial evidence implicates the eosinophil as a major effector cell in various diseases of the lung. Eosinophils can release preformed and *de novo* generated mediators, the actions of which may invoke many of the pathological features, not only of asthma but also of acute and chronic interstitial lung disorders. The underlying immune mechanism could be initiated by exogenous antigens, fig. 8 ①, inducing antigen presenting cells to release inter-

leukins 4, 5 and 6 (IL-4, IL-5, IL-6) and interferon- γ (IFN- γ), ②, thereby regulating proliferation and differentiation of T- and B-lymphocytes ③. Plasma cells may produce immunoglobulin-E (IgE), which binds to tissue dwelling mast cells and infiltrating basophils, ④. T-lymphocytes may further differentiate into the Th2 subset, which produces a defined spectrum of proinflammatory cytokines, ⑤.

After being released from the bone marrow into the circulation, ⑥, under the influence of cytokines (granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3 and IL-5) possibly derived from antigen-stimulated T-lymphocytes, mast cells, platelets, or interleukin-1 (IL-1)- and tumour necrosis factor (TNF)-stimulated endothelium, ⑥, eosinophils may undergo priming and augment the membrane expression of adhesion molecules such as CD 11b, ⑧. At the same time, tissue mast cells, previously sensitized by plasma cell-derived IgE, bind specific antigen, triggering the release of bioactive mediators into the bronchial mucosa and lumen, and thereby inducing the early allergic response. The concerted action of cytokines and PAF secreted by mast cells and other inflammatory cells may then induce eosinophil adhesion to endothelial cells, ⑨, diapedesis or penetration through the blood vessel wall, ⑩, and the migration of the eosinophil into the affected tissue, ⑪. After arrival at the inflammatory focus, the eosinophil may at first be immobilized and hyporeactive, due to cell desensitization mediated by

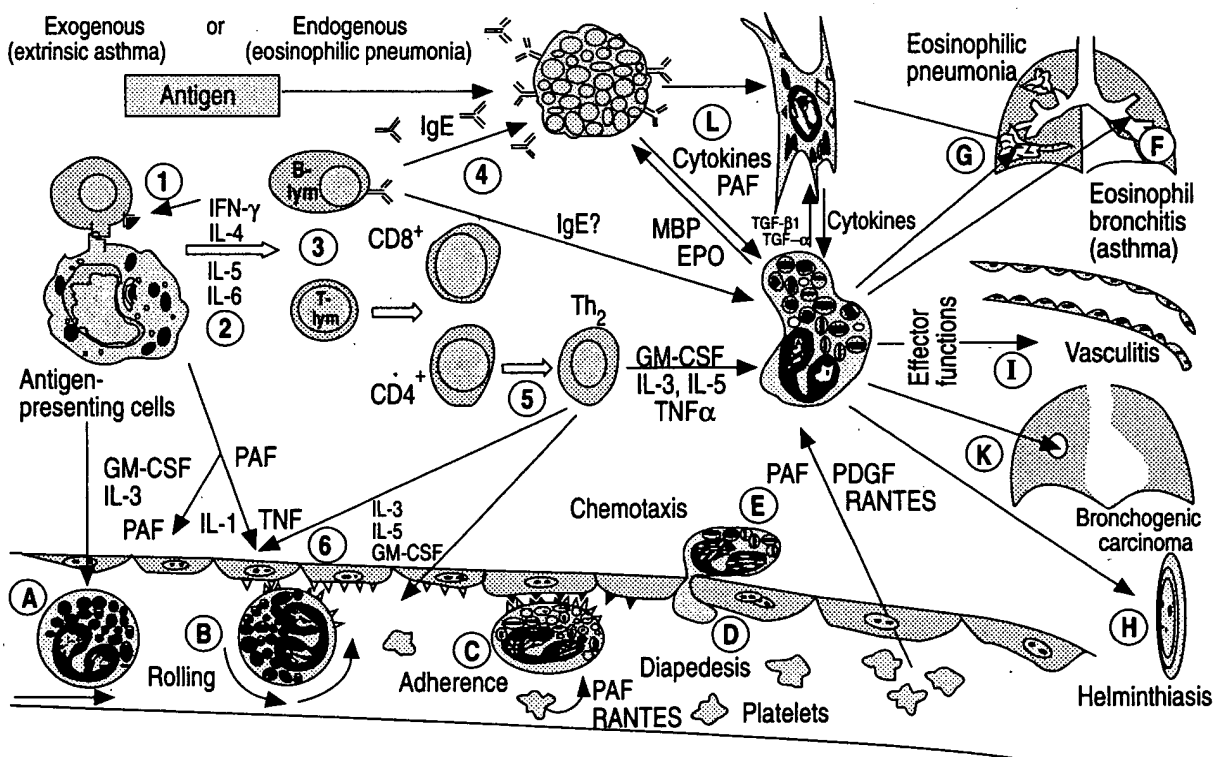


Fig. 8. – Putative mechanisms of the eosinophil-dominated inflammatory reaction. For details see text. B-lym: B-lymphocytes; EPO: eosinophil peroxidase; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN- γ : interferon- γ ; IgE: immunoglobulin E; IL: interleukin; MBP: major basic protein; PAF: platelet-activating factor; TNF: tumour necrosis factor; TGF: tumour-growth factor; T-lym: T-lymphocytes; PDGF: platelet-derived growth factor.

chemotactic factors such as PAF. The continued exposure of the cell to locally secreted cytokines, however, may enhance survival and prime the cell, enabling the eosinophil to regain its proinflammatory properties, namely the release of granule proteins, cytokines, lipid mediators and reactive oxygen species [138]. Depending on the respective antigen, the infiltrated tissue and its histological properties and physiological function, eosinophil-derived products may either cause destruction of airway epithelium (bronchial asthma), \oplus , interstitial destruction and oedema (eosinophilic pneumonia, parasitic infestation), \odot and \oplus , or granulomatous tissue destruction in certain vasculitic disorders (Churg-Strauss syndrome), \odot . Toxic products secreted by the eosinophil may even contribute to the host defence mechanism against bronchogenic carcinoma, \oplus . In addition, eosinophil-derived basic proteins and lipid mediators, together with cytokines, may enhance the function of surrounding tissue dwelling and infiltrating cells, \odot , thereby further intensifying the local inflammatory tissue reaction.

Table 7. - List of eosinophil-associated respiratory diseases

Infectious diseases	
	Tissue-invasive helminths
	Filariasis
	Schistosomiasis
	Strongyloides
	Trichinosis
	Toxocariasis
	Ascariasis
	Echinococcosis/cysticercosis
Other infections	
	Acute coccidioidomycosis
	Afebrile tuberculosis
	Cat scratch disease
	Chlamydial pneumonia of infancy
Interstitial and other pulmonary diseases	
	Transient pulmonary eosinophilic infiltrates (Löffler)
	Chronic eosinophilic pneumonia
	Hypersensitivity pneumonitis
	Allergic bronchopulmonary aspergillosis
	Topical eosinophilia
	Idiopathic pulmonary fibrosis
	Sarcoidosis
	Histiocytosis X
	Eosinophilic pleural effusions
Vasculitic granulomatous diseases	
	Churg-Strauss syndrome
	Temporal vasculitis
	Wegener's granulomatosis
	Polyarteritis nodosa
Immunological diseases	
	Graft rejection
	Intrinsic bronchial asthma?
Allergic disorders	
	Allergic rhinitis
	Extrinsic bronchial asthma
	Drug reactions
Neoplastic and myeloproliferative diseases	
	Lymphomas, especially T-cell type and Hodgkin's disease
	Bronchogenic carcinoma
	Hypereosinophilic syndrome

From [2] after modification.

Role in human disease

Eosinophils have been implicated in a wide spectrum of human disease (table 7), ranging from parasitic infestations and allergic reactions to vasculitic and granulomatous diseases [2, 326, 351–368]. The potential role of the eosinophil in helminthic infections, interstitial lung disorders, and bronchial asthma, including both their beneficial or protective and detrimental role, will be outlined below.

Parasitic infestations

The association between helminthic infestation and peripheral blood eosinophilia dates back to the beginning of this century, and frequently involves the lung (tropical filarial eosinophilia) [359]. Four main findings have emerged over the past two decades. Firstly, anti-eosinophil serum reduces both the number of peripheral blood eosinophils and increases susceptibility to parasites [325]. Secondly, eosinophils are directly involved in the killing of helminths [323, 360]. Thirdly, eosinophil granule proteins and oxygen metabolites are cytotoxic for parasites [361, 362]. Finally, eosinophils accumulate and degranulate around parasites *in vivo* [363, 364]. These data appear to support a role for the eosinophil in host defence against parasites.

More recently, however, studies investigating the role of cytokines [365, 366] on the immunity to helminths in mice seem to contradict the above mentioned findings. Administration of anti-IL-5 and anti-IL-4 antibodies markedly reduced blood and tissue eosinophilia as well as serum IgE levels, but failed to diminish immunity to the parasite. In contrast to these findings, treatment with antibodies to IFN- γ caused partial depletion of immunity against the schistosomula in the lungs [367]. Although mice may employ different immune mechanisms for parasite resistance, the apparent discrepancy with previous work clearly warrants further clarification.

The mechanism whereby eosinophils destroy parasites can be divided into recognition, attachment and killing phases. Recognition is facilitated by chemotactic factors produced by other participating inflammatory cells and by parasite-derived factors. In addition, immunoglobulin G (IgG) antibodies [368] and complement proteins [369], facilitate the attachment of the eosinophil to the parasite. Adhering eosinophils flatten and release granule proteins [370, 371], and oxygen radicals [99, 108, 372], onto the surface of the parasite eventually leading to its destruction.

Interstitial lung disorders

Although eosinophils are rarely found in the normal human lower respiratory tract, accumulation of eosinophils in the parenchyma is found in the course of various inflammatory diseases [373, 374]. Besides the well-known eosinophil-associated histiocytosis-X [375], and chronic eosinophilic pneumonia [376], other diseases,

such as idiopathic pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, and chronic interstitial disease associated with the collagen-vascular disorders can, on occasion, also be characterized by an accumulation of significant numbers of eosinophils in alveolar structures and the interstitium [355, 356]. The reasons for this inconsistent observation of eosinophil involvement in these diseases may be related to disease variability, as well as early treatment with corticosteroids in these patients.

Although neutrophils are considered to be the major effector cell in idiopathic pulmonary fibrosis (IPF), there is evidence that eosinophils may at least contribute to the underlying inflammatory process in ongoing disease. Eosinophils are normally found in the bronchoalveolar lavage (BAL) fluid, and in approximately one third of the patients, the eosinophil number exceeds that of neutrophils [377]. In addition, eosinophils have been associated with progression of the disease [377, 378]. The levels of ECP in BAL fluid from patients with IPF are significantly increased compared to healthy controls, and its concentration correlates with a reduction in diffusion capacity of the lung [379]. Similarly, increased ECP levels in BAL fluid are also associated with severity of lung damage in patients suffering from adult respiratory distress syndrome (ARDS) [380].

Although the cause of eosinophil accumulation in some of these disorders is unknown, increasing evidence suggests that the eosinophil can function as an effector cell capable of mediating direct interstitial tissue destruction. A role for eosinophils in mediating injury to the lung parenchyma in ARDS is supported by experiments demonstrating that the cells are cytotoxic to several types of lung parenchyma cells *in vitro*, and that they can degrade matrix components of the human lung parenchyma [326, 328]. In general, two different mechanisms may be involved. Firstly, the eosinophil collagenase has been shown to specifically cleave human lung collagens type I and III [326, 381]. Secondly, eosinophils damage human lung parenchyma cells through their granular basic proteins and the generation of reactive oxygen radicals. For instance, eosinophils are cytotoxic for lung fibroblasts, mesothelial cells, and epithelial cells [325, 326, 328, 330, 382]. Eosinophil-mediated cytotoxicity could be partially inhibited by anti-oxidants [253, 330], suggesting a role for both oxidative and nonoxidative effector mechanisms.

Eosinophilic pneumonia

The eosinophilic pneumonias comprise a broad spectrum of disorders clinically characterized by systemic illness, severe constitutional symptoms, and mainly peripheral pulmonary infiltrates on the chest X-ray film [355, 376, 383]. The common denominator of these syndromes is an infiltrative eosinophilia [384], accompanied by an increased number of eosinophils in blood and BAL [148, 154, 265, 385, 386]. Treatment with corticosteroids often leads to rapid recovery, but prolonged therapy is frequently required to avoid recurrence [383, 387, 388].

Although the pathogenesis of eosinophilic pneumonia is not yet understood, increasing evidence suggests that the eosinophil may represent a major effector cell in the underlying pathomechanisms of the disease. This conclusion is based on several studies of lung tissue and BAL fluid from patients with eosinophilic pneumonia, showing increased concentration of ECP in the BAL fluid [265], localization of eosinophil granule MBP in areas of eosinophilic microabscesses [327, 388], numerous lysed eosinophils [265], and free granules within the pulmonary microvasculature, as well as deposits of MBP in parenchymal lesions [389–392]. In addition, bronchoalveolar eosinophils obtained from these patients show extensive degranulation [154, 265], are predominantly hypodense [37, 148, 154, 265], and express several activation markers, including intercellular adhesion molecule-1 (ICAM-1), CD 11b, as well as the major histocompatibility class II antigen, HLA-DR [37, 140], suggesting a profound activation of pulmonary eosinophils in this disease.

The above observations are complemented by *in vitro* studies, which show that eosinophil-derived granular proteins can directly injure pulmonary endothelial cells, increase the transvascular flux of proteins across endothelial monolayers, and cause lung oedema in isolated perfused rat lungs [393, 394]. Hence, eosinophils may be directly responsible for the tissue injury in eosinophilic pneumonia, including the variable infiltrates of the lung. In addition, since the class II protein HLA-DR mediates the interaction of accessory cells with CD4⁺ lymphocytes, it may be conceivable that eosinophils function as an antigen-presenting cell for an, as yet unknown, antigen in eosinophilic pneumonia.

Vasculitic granulomatous diseases

Deposits of eosinophil granule products have been demonstrated in a number of vasculitic granulomatous disorders, such as Churg-Strauss syndrome [395], necrotizing vasculitic lesions in polyarteritis nodosa and Wegener's disease [396], systemic vasculitis of unknown origin [397], and in temporal arteritis [398]. Deposits of eosinophil-derived proteins in inflamed vessels are preferentially found in areas with necrotic lesions and in thrombi. In contrast, eosinophils or their products can not be found in atherosclerotic arteries, suggesting that involvement of eosinophils in vasculitic granulomatous disease is specific.

Graft rejection

Experimental lung allograft in the rat causes eosinophil infiltration, accounting for up to 20% of the cellular infiltrate within 4 days after implantation [399]. In man, several reports have demonstrated that eosinophils may also participate in graft rejection, although it is not yet clear whether the eosinophils actively participate in the underlying immune response or whether they simply represent a nonspecific marker for lymphocyte activation.

However, eosinophilia occurring after renal transplantation was shown to be an adverse prognostic factor for graft survival [400–402]. In addition, an increase in the number of blood and graft eosinophils was reported to be a sensitive and specific indicator for acute rejection [403].

Eosinophilic pleural effusion

Eosinophils are often observed in pleural effusion associated with pneumothorax, asbestosis, pulmonary infarction, sarcoidosis and collagen vascular disease [404, 405]. In addition, eosinophils occur in pleural effusion of unknown cause. Although the pathogenic significance of pleural eosinophils is not yet clear, their presence appear to reduce the likelihood of an underlying malignant disease or pulmonary tuberculosis [404–408]. Recent reports have shown that eosinophilic pleural effusions contain significant concentrations of IL-5, GM-CSF and interleukin-3 (IL-3) and that these factors contribute to eosinophil proliferation and survival [225]. In addition, administration of interleukin-2 (IL-2) into the pleural cavity has been shown to cause eosinophil accumulation indirectly *via* the release of these cytokines by lymphocytes [225].

Bronchial asthma

Bronchial asthma has been defined as a lung disease with reversible airway obstruction, airway hyperresponsiveness and airway inflammation [409]. The disease occurs as an intrinsic and an extrinsic, atopy-related form. Although different immunological pathomechanisms may be operative [410], both types have in common an eosinophil-dominated bronchial inflammatory reaction of the bronchial tissue [308, 411–417]. Eosinophils or their granular products are preferentially found along the lining of bronchioles close to the areas of damaged epithelium and mucous plugs [80, 308, 351, 352, 418, 419]. An increase in the number of bronchial epithelial eosinophils during exacerbation of naturally occurring asthma is associated with an increase in both airway hyperresponsiveness and asthma symptoms [308].

Immunohistological analysis of bronchial tissue has provided additional evidence that eosinophils are actively involved in the inflammatory tissue reaction in asthmatic airways [308, 352, 418]. Histological sections of lungs from patients with asthma show MBP deposited along the lining of bronchioles close to the areas of damaged epithelium and mucous plugs [351, 352]. Immunofluorescence studies with two mouse monoclonal antibodies EG1 (specific for ECP) and EG2 (specific for a common epitope of secreted ECP and eosinophil-derived neurotoxin (EDN)) have demonstrated that degranulated eosinophils were found beneath the basement membrane and among epithelial cells, even in patients with mild asthma, where degranulation was related to epithelial damage [352].

Besides their occurrence in bronchial tissue, eosinophils may also be present in peripheral blood obtained

from asthmatic subjects [352, 420–423]. In addition, an inverse correlation between blood and eosinophil count and the degree of bronchial hyperresponsiveness expressed in methacholine provocation concentration producing a 20% fall in forced expiratory volume in one second (PC_{20}) [420, 424, 425] has been reported. A correlation was also found between the number of blood eosinophils and the forced expiratory volume in one second (FEV_1) in subjects with intrinsic asthma [425], and in atopic asthmatics with the late allergic response [426]. Moreover, a study on the effect of immunotherapy on bronchial responsiveness in pollen-allergic patients with a history of rhinoconjunctivitis and wheezing suggests that there is a correlation between serum ECP concentration and bronchial hyperresponsiveness [354]. In untreated patients, the level of ECP increased significantly during the pollen season, but this did not occur in patients receiving immunotherapy.

Large numbers of eosinophils or eosinophil-derived proteins are found in sputum of symptomatic asthmatics, particularly in aspirin-induced asthma [253, 329, 427–429]. Compact clusters of columnar cells containing numerous eosinophils, eosinophil granules as well as Creola bodies may also be seen [416]. In addition, it has been demonstrated that the concentration of MBP in sputum is consistently raised and the detection of this protein is specific for asthma [329, 428]. The amount of Creola bodies and EPO in sputa were also elevated in asthma, although these proteins are not specific for this disease [253].

Increased numbers of eosinophils are also found in BAL fluid from asthmatics [310, 352, 417, 430–435]. In stable, nonsymptomatic asthmatics or in corticosteroid treated subjects, the proportion of eosinophils, however, is not excessively increased, and may even be normal [433, 436]. In addition, eosinophil count may differ depending on the underlying form of asthma. For instance, comparison of different asthmatic types have demonstrated that the blood eosinophil count may be higher in atopic than in nonatopic asthmatics, and even greater in patients with aspirin-sensitive asthma [437].

Eosinophil-derived basic proteins have been shown to cause direct damage to both guinea-pig and human respiratory epithelium [351]. At low doses, MBP causes exfoliation of epithelial cells and impairment of ciliary beating. At higher concentrations, MBP detaches ciliated and brush cells and destroys individual cells exposing the basal cell layer. At an ultrastructural level, MBP disrupts the plasma membrane, liberating the cellular contents [253, 325, 351, 416, 438]. Examination of asthmatic bronchial tissue sections by immunofluorescence technology shows deposition of MBP at the sites of epithelial damage [351]. ECP also caused a dose-related damage to guinea-pig tracheal epithelium, as assessed by inverted microscopy. In addition, EPO at low concentrations, either alone or in the presence of hydrogen peroxide and a halide, caused ciliostasis, bleb formation, and exfoliation of epithelial cells [253]. Finally, PAF-activated eosinophils also led to ciliostasis and disruption of respiratory epithelium *in vitro* [330]. Taken together, these observations strongly suggest that

the eosinophil represents an effector cell in asthma capable of damaging respiratory epithelium.

Airway challenge in allergic subjects has been used as an experimental model to study physiological responses and development of airway inflammation. With the more widespread use of fiberoptic bronchoscopy as an investigative tool in asthma, an increasing number of studies have shown that local endobronchial allergen provocation with subsequent performance of BAL represents a new approach in studying allergen-driven airway inflammation which is representative of naturally occurring asthma (for review see [436]). These studies have convincingly demonstrated a consistent increase in eosinophil numbers recovered by BAL in the absence of provocation. In addition, when mild asthmatics were challenged with antigen in a subsegment bronchus, a multifold increase in total cell recovery compared to a control sham-challenged segment was observed after 19–96 h [43, 138, 153, 432, 436]. This rise in cell numbers was due mainly to an increase in numbers of eosinophils. There was a less pronounced but significant rise in lymphocytes and basophils as well as mast cells, whereas the numbers of neutrophils, macrophages and epithelial cells did not differ significantly between the antigen and control segment [36, 37, 43, 138, 153, 163, 432, 436]. In contrast, no change in either the differential count or the absolute cell count was observed in the antigen or control sites 10 min after challenge. The results clearly demonstrate that antigen challenge of airways of even mild asthmatic subjects induces an inflammatory cell recruitment that is numerically predominated by eosinophils, and to a lesser extent by lymphocytes, mast cells and basophils.

Bronchogenic tumours

The association of eosinophilia and malignancies was first described in 1893 in a 31 year old woman with a tumour of the neck [439]. Since then, a number of reports have been published describing either increased eosinophil counts or infiltration in lymphoreticular malignancies, lymphocytic leukaemias, intestinal tumours and carcinoma of the lung (for review see [2]). However, expression of eosinophilia both in peripheral blood and tumour tissue varies significantly, and only a small proportion of patients with oral, gastric and breast carcinomas show increased eosinophils within the tumour tissue. In contrast, in both carcinoma of the cervix and colon [440], eosinophils were observed in approximately one third of the cases. Even more important, a positive correlation was found between tumour eosinophils and the survival rate [440].

In lung cancer, only a few studies have been published to date. In a study of 72 operable primary lung cancers, 59% [37] showed a prominent local infiltration of eosinophils. Follow-up studies indicated that eosinophil tumour infiltration was associated with a good prognosis, whilst the absence of eosinophils indicated a poor outcome [441, 442]. In a recent prospective study on

720 consecutive patients, the number of eosinophils as well as the level of serum ECP was raised not only in patients with asthma, hypersensitivity pneumonitis, and bronchiectases, but also in patients with carcinomas of the lung [443]. Since ECP is a better indicator of eosinophil involvement than eosinophil cellular counts, these data strongly support eosinophil involvement in bronchogenic tumours. Whether ECP or other eosinophil-derived proteins may prove to be a useful soluble tumour marker warrants further investigation.

Although the significance of eosinophils in bronchogenic tumours is far from understood, eosinophils may be part of the immunological anti-tumour response of the host. As mentioned above, eosinophil-derived proteins have been shown to kill tumour cells *in vitro* [2, 350]. In addition, tumour patients which responded to radiation therapy with a blood eosinophilia [444, 445] showed double the median survival of those who did not [445]. Also, the incidence of naturally-occurring mammary tumours in mice was reduced when they developed an eosinophilia following infection with *Trichinella spiralis* [446]. Furthermore, development of sarcoma was completely suppressed in rats infected with *Nippostrongylus brasiliensis*, five days before tumour inoculation [447]. When tumour cells secreting IL-4 or IL-2 were introduced into mice, the tumours showed an eosinophilic infiltrate and were rejected faster than in animals with nonsecreting tumours [288]. In addition, the studies demonstrate that the cytokine-associated tumour cytotoxicity was dependent on eosinophils. Thus, it may be concluded that eosinophils may contribute to the host antitumour response. Further studies investigating the role of eosinophils in lung cancer are currently under way.

Therapeutic perspectives

Several drugs commonly used for asthma treatment have been shown to affect eosinophil functions (table 8). However, the increased knowledge of eosinophil cellular biology and effector function in the context of an inflammatory lymphocyte-guided reaction during recent years has prepared the ground for the development and rational use of several other classes of drugs. These include modern antihistamines with anti-inflammatory action, immune response modifiers, anticytokine and antimediator antibodies, as well as synthetase inhibitors, antioxidants and protein neutralizers. The final section of this paper aims to review the currently available anti-eosinophil drugs, with a special emphasis on substances still in early clinical development.

Corticosteroids. Corticosteroids are the most effective anti-inflammatory drugs so far known for treating many eosinophil-related disorders. Corticosteroids are likely to have a multifactorial therapeutic effect, resulting from the suppression of the regulatory mechanisms of the immune response, migratory action, as well as the distal effector functions of eosinophils. For instance, corticosteroids reduce the number of sputum [448], and

peripheral blood eosinophils [152, 410, 449–451], presumably through a decreased release from the bone marrow [452], a reduction in eosinophil survival [453, 454], and inhibition of eosinophil tissue infiltration probably by inhibiting both eosinophil chemotaxis [455, 456] and adherence to endothelium [47, 457–459]. Corticosteroids also diminish antibody-dependent eosinophil cytotoxicity [460]. In asthmatics, inhaled steroids reduce the concentration of ECP in serum and sputum [414, 424, 461, 462], as well as in BAL fluid [463]. In addition, normal eosinophil counts were found in the BAL fluid of patients undergoing a steroid therapy [463], which may be due, in part, to inhibition of GM-CSF production by bronchial epithelial cells [292, 293]. Steroids also diminish the number of circulating hypodense eosinophils [152, 464], and inhibit the generation of superoxide anions by eosinophils when administered to normal subjects [465].

In addition, steroids have been shown to inhibit eosinophil activation *in vitro*. Several studies have demonstrated that corticosteroids prevented the expression of Fc receptors [466], degranulation [467], chemotaxis and adherence [456, 457], the formation of lyso-PAF, leukotrienes, and 15-hydroxyeicosatetraenoic acid (15-HETE) [468], and the release of EPO and superoxide anions [469].

Eosinophils express glucocorticoid receptors [470], and in one report their absence was correlated with clinical glucocorticoid resistance in the hypereosinophilic syndrome [471].

β -agonists. β_2 -agonists are the most effective bronchodilators in current use, and act predominantly by relaxing airway smooth muscle, but also by inhibiting mast cell degranulation [472]. In passively sensitized guinea pigs, inhalation of β_2 -stimulants at low concentrations (0.004–0.3%) inhibited the increase in the number of eosinophils in the BAL fluid after allergen challenge [473]. In humans, treatment with β_2 -agonists and theophylline decreases the serum ECP level [461]. Furthermore, β_2 -agonists reduced the release of both EPO and superoxide anions from zymosan stimulated human eosinophils *in vitro* [474]. The effect of these drugs are mediated through activation of β_2 -receptors which, in turn, are coupled to adenylate cyclase [475, 476]. It is interesting that the magnitude of inhibition was associated with a short preincubation time indicating that desensitization of β -receptors may occur rapidly after exposure to the agonist.

Xanthines and selective phosphodiesterase inhibitors. Xanthines, such as theophylline, are bronchodilating drugs in the management of asthma. Although it has been suggested that theophylline has, in addition, some anti-inflammatory action [477], its effects on eosinophil function is yet to be established. Purified guinea-pig and human eosinophils respond to preincubation with theophylline in a biphasic manner: at therapeutic concentrations theophylline enhanced the response of the cells stimulated with opsonized zymosan. Only high concentrations (10^{-3} M) were inhibitory [475]. This

Table 8. – Effects of various drugs on cellular functions of the eosinophil leucocyte

Eosinophil function	Anti-eosinophil drug
Tissue infiltration	Corticosteroids Ketotifen DSCG PDE type IV inhibitors Cetirizine PAF receptor antagonists Anti-IL-5 receptor antagonists
Chemotaxis	Corticosteroids Nedocromil PDE type IV inhibitors? Cetirizine PAF receptor antagonists
Surface receptor expression	Corticosteroids Nedocromil Cetirizine
Adhesion	Corticosteroids Cetirizine
Hypodensity	Corticosteroids Nedocromil Azelastine
Survival	Corticosteroids
Granular protein secretion	Corticosteroids Nedocromil DSCG Ketotifen β_2 -agonists
Oxygen radical production	Corticosteroids DSCG β_2 -agonists
Lipid mediator release	Corticosteroids Nedocromil
Cytotoxicity	Corticosteroids Nedocromil Cetirizine

DSCG: disodium cromoglycate; PDE: phosphodiesterase; PAF: platelet-activating factor; IL-5: interleukin-5.

observation may explain the lack of effect of theophylline on bronchial hyperreactivity [478].

Previous studies have established that guinea-pig peritoneal eosinophils contain at least one type IV phosphodiesterase (PDE) isoenzyme [479, 480]. In an animal model, treatment with the type III/IV selective PDE inhibitor, zardaverine [481], significantly reduced the influx of eosinophils in the peritoneum following serum-protein challenge [482]. In addition, eosinophils purified from the treated animals showed a significant reduction of actin polymerization and diminished chemotactic migration in response to both PAF and C5a *ex vivo*. The clinical significance of selective PDE inhibitors awaits to be elucidated.

Disodium cromoglycate. Disodium cromoglycate (DSCG) has been widely used in the treatment of bronchial asthma, and appears to be particularly useful for atopic asthmatic subjects. DSCG treatment significantly reduces the number of eosinophils in bronchial mucus and BAL fluid [417]. In addition, it has been shown to inhibit the tissue accumulation of eosinophils during

the late response to allergen in IgE-sensitized rabbits [483], and to PAF in guinea-pig [484] and human skin [485], as well as eosinophil activation *in vitro* [486, 487].

Nedocromil sodium. Nedocromil sodium is a pyranoquinoline dicarboxylic acid which has been demonstrated to have some beneficial effects in various models of asthma, including human airway response to hyperosmolar challenge [488], PAF-mediated bronchoconstriction [489], early and late responses to allergen challenge in asthmatics [490, 491], and eosinophil recruitment in humans [491]. Besides its inhibitory action on mast cells, neutrophils, monocytes and platelets [492–496], nedocromil sodium has been shown to block various eosinophil functions, including secretion of granule proteins [497], eosinophil chemotaxis [498, 499], surface antigen expression [500], leukotriene C₄ secretion in response to various agents [499, 501] as well as density change, damage to airway epithelium [502, 503] and other cytotoxic mechanisms [498, 500]. Hence, these inhibitory effects of nedocromil on eosinophil function suggest that it may be effective in eosinophil-related disease, such as asthma.

Ketotifen. Ketotifen is a benzocycloheptathiophene with anti-allergic properties as demonstrated in a variety of *in vivo* and *in vitro* models [504]. Besides its ability to block the action of histamine, the release of mediators from mast cells, calcium uptake and to prevent and reverse β -adrenergic tachyphylactic reactions, ketotifen has been shown to inhibit eosinophil degranulation [505], and the recruitment of eosinophils into the airways that results from exposure to PAF [506].

Cetirizine. Cetirizine is a second generation, non-sedating highly selective H₁ antihistamine, which has been shown to be effective in the prophylaxis and treatment of asthma [507–510]. In addition to its anti-H₁-receptor properties, cetirizine possesses inhibitory properties for basophils [511], and eosinophils [512–514]. For instance, cetirizine has been shown to inhibit chemotaxis of eosinophils *in vivo* [512, 513, 515], and *in vitro* [514], reduces both PAF-induced receptor complement expression and cytotoxicity [516], prevents IL-5-induced ICAM-1 expression *in vitro* [517], and selectively blocks adhesion of eosinophil to endothelial cells [518] possibly *via* the very late activation antigen-4 (VLA-4)/vascular cell adhesion molecule-1 (VCAM-1) interaction.

Azelastine. Azelastine is a phthalazinone derivative with anti-allergic and anti-asthmatic properties [504]. Besides its potent antihistamine action, it also has inhibitory effects on oxygen radicals released by neutrophils, macrophages, and eosinophils [519]. However, for the other new antihistamines, the mechanism of action for azelastin is unknown.

PAF-antagonists. Over the past 10 yrs, a number of antagonists for PAF have been developed (for review see [520]), such as WEB 2068, MK-287 and UK-74,505.

Their potent inhibitory effect on eosinophil function has been shown *in vitro* [64, 72, 194] and in antigen-induced airway responses in humans [521–525]. However, in some clinical trials, the effect of PAF receptor antagonists given orally or by inhalation has been disappointing [526–529].

The reasons for this discrepancy are currently not understood. However, several explanations may be considered. Firstly, it may prove difficult to effectively block the effect of endogenously released PAF, since PAF appears to be a mediator which to a large extent remains associated with cells and has almost a "paracrine" effect influencing only neighbouring cells. It may, therefore, interact with other cells at rather high concentrations that may be difficult to antagonize with currently available competitive antagonists. Perhaps, even more potent antagonists must be developed, or drugs which specifically block the synthesis of PAF. Alternatively, higher local concentrations may be achieved by inhalation of an antagonist.

Secondly, PAF comprises a family of multiple molecular species, including both saturated and unsaturated 1-O-alkyl homologues, 1-O-acyl analogues, and acetylated phosphoglycerides having polar head groups other than choline. Different molecular species of PAF can be produced by an inflammatory cell. The biological significance of the molecular heterogeneity is not yet clear. However, given the variable biological activity of each member of the PAF family [530], the inflammatory cell may be able to alter the predominant "type" of PAF, thereby determining the degree of inflammatory activity. Furthermore, PAF may use not only different molecular species with different biological activities, but also different receptor subtypes with various affinity states and different signalling mechanisms to differentially regulate pathophysiological processes, for instance in bronchial tissue in asthma [531]. Hence, this heterogeneity may, in part, explain the somewhat conflicting results regarding the effectiveness of PAF receptor antagonists, and suggest that the action of currently available antagonists may not be sufficient to block total PAF activity. More potent PAF receptor antagonists with a different selectivity profile and both intracellular and extracellular action may prove more beneficial [532].

Leukotriene synthesis inhibitors and receptor antagonists. Sulphidopeptide leukotrienes (LT) had already been identified in the late 1930s by Kellewy and Trethewie, and have since been suggested as playing a role in allergic airway disease by mediating smooth muscle contraction, bronchial secretion of mucus, and airway mucosal oedema by increasing postcapillary venopermeability [533, 534]. In addition, a recent study suggests that inhalation of LTE₄, the most stable of the sulphidopeptide leukotrienes, elicits eosinophil recruitment in human airways [535]. Sulphidopeptide leukotrienes, LTC₄, LTD₄, and LTE₄, provoke airway obstruction *via* binding to the LTD₄ receptor, since separate LTC₄ or LTE₄ receptors have not been identified [536]. Eosinophils are a major source for LTC₄ and

may contribute to LT-elicited airway response during the late reaction [63, 115–117].

The development of the leukotriene D receptor antagonists has confirmed the relative contribution of these specific cellular products in allergic disease, such as asthma. Indeed, clinical and experimental studies employing several LTD₄ receptor antagonists suggest a beneficial effect of these substances in allergic asthma [537–541], exercise-induced asthma [542–545], aspirin-induced asthma [546], PAF or antigen-mediated bronchoconstriction [547–549], as well as *Ascaris*-induced late-phase bronchoconstriction, airway responsiveness, microvascular leakage, and leucocyte infiltration [550, 551]. However, since binding sites for sulphidopeptide leukotrienes on eosinophils have not been found, a direct effect of leukotriene D receptor antagonists on eosinophils is unlikely.

Thromboxane synthetase inhibitors and antagonists. TxA₂, PGD₂ and PGF_{2α} are short-lived but highly potent smooth muscle contractors in man, and have been demonstrated to be released by human and guinea-pig eosinophils [63, 119]. The eicosanoids mediate their biological action *via* binding to the thromboxane receptors, which are widely distributed in airway smooth muscle. Hence, conceptually thromboxane receptor antagonists, such as GR32191 or ICI 92605 may be more effective than thromboxane synthetase inhibitors which do not prevent synthesis of PGD₂ or PGF_{2α}. While their action on eosinophils and other immune cells remains to be elucidated, preliminary clinical trials have been promising [552].

Cytokine antagonists and cyclosporin. Since cytokines may play a crucial role in mediating eosinophil tissue infiltration, the administration of cytokine antagonists may be useful in eosinophil-associated disease. This view is supported by animal experiments showing that monoclonal anti-IL-5 antibodies suppress blood eosinophilia and infiltration of eosinophils into the lungs of mice parasitized with *Nippostrongylus brasiliensis* [367, 553, 554]. In view of the capacity of GM-CSF to render eosinophils resistant to the effect of corticosteroids [555], the concurrent administration of steroids with a cytokine receptor blocker may augment their effectiveness.

A similar approach to modifying the elaboration of eosinophilopoietic cytokines may be achieved by other drugs, such as cyclosporin A. Cyclosporin A has been successfully used as an immunosuppressant in organ transplantation. It is thought to inhibit proliferation of T-lymphocytes and cytokine release [556], and may also affect immune effector cells, such as mast cells, basophils or eosinophils *via* interaction with cyclophillin [557]; and unpublished results. In an experimental study using T-cell clones from a patient with hyper-eosinophilic syndrome (HES) as a model target system, it could be demonstrated that cyclosporin A induced a clinical remission by abolishing the generation of these cytokines [558]. In a clinical randomized cross-over study on 33 patients with severe chronic corticosteroid-

dependent asthma [559], administration of cyclosporin A improved the lung function and reduced the number of exacerbations in the asthmatics. Although its mode of action remains to be elucidated in asthma, it is likely that cyclosporin A modifies the lymphocyte driven eosinophil response.

Antioxidants and protein neutralizers. Oxygen radicals play an important role in eosinophil-mediated epithelium toxicity [253, 330]. Hence, detoxification of toxic oxygen metabolites may be a useful approach to prevent inflammatory tissue damage. N-acetyl-L-cysteine is widely used as a mucolytic but it may also act as an antioxidant, as a reducing agent and as a chelating agent. Thus, it may neutralize toxic oxygen radicals and other oxidative mediators of inflammation released by eosinophils and other immune cells. However, whilst it lessens the toxic effects of paracetamol (acetaminophen) overdose, its efficiency in inflammatory conditions is uncertain [560–562], and awaits further investigation.

As has been mentioned above, heparin and related anionic molecules released from mast cells and basophils, neutralizes the toxic properties of MBP on various cells [252–254]. Hence, administration of the highly anionic heparin as an aerosol may reduce the toxic effects of eosinophil-derived basic proteins on airway epithelium. In an animal model, heparin reduced the epithelial damage of guinea-pig caused by MBP [252, 253]. In addition, heparin has been shown to inhibit eosinophil infiltration into lung tissue of laboratory animals [563], which may be related to its effects on lymphocyte activation [564] endothelial permeability [565], and negative charge [566], or *via* neutralization of eosinophil chemotactic factors itself [567]. Finally, heparin may modulate the release of proinflammatory mediators to prevent exercise-induced asthma [568].

A similar, yet more experimental, approach to neutralize basic proteins employing other acidic substances has only recently been suggested. For instance, acidic polyamino acid has been shown to inhibit the toxicity of MBP and ECP to cultured K562 cells and guinea-pig tracheal epithelium [332, 333]. In addition, aerosol inhalation of an acidic polyglutamic acid in primates significantly inhibited the MBP-induced increase in airway resistance and hyperresponsiveness as assessed by methacholine.

Perspective

The last 10 yrs have seen a tremendous increase in our understanding of the pathophysiological role of eosinophils, and the pace of research still seems to be accelerating. So far, several aspects of the immunobiology of the eosinophil have been outlined. These include cellular functions and survival, signal transduction, the role of cytokines on eosinophil differentiation, cytotoxic capacity, the molecular biology and mode of action of the eosinophil granule proteins, as well as their recruitment from the circulation into the

tissues. However, numerous important questions remain to be addressed. Whilst most of the current knowledge on eosinophils has been obtained from allergic and asthmatic patients, little is known of their role in other diseases, such as eosinophilic pneumonia, vasculitic/granulomatous disorders and even malignancies. It may be possible to fortify the host antitumour immune response by raising eosinophil cytotoxicity. In addition, the molecular biology of the eosinophil as a source of cytokines or other proteins has to be further elucidated. The question of whether cellular hypodensity may be restricted to the eosinophil, or whether it may reflect a general reaction of inflammatory cells following activation deserves further attention. Work on the nature of the immune reaction leading to tissue eosinophilia, i.e. the association with IgE-related allergic reactions as opposed to nonallergic disorders, represents another important perspective. In this context, understanding of the pathogenesis of intrinsic asthma may provide further insight into this question.

IL-5 may be an important mediator responsible for eosinophil tissue infiltration. However, other factors may also be necessary and future work will have to delineate the differential effects of various eosinogenic mediators. In addition, the possible benefits of the application of anti-adhesion molecules or anti-cytokines, as well as other antagonists, *in vivo* will further help to define their respective significance for eosinophil activation. Finally, a number of drugs with potential anti-eosinophil properties have been developed. Studies are under way to characterize their effects on eosinophils in the context of eosinophil-dominated diseases. There is still a quest for drugs capable of selectively modifying eosinophil function.

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